116

FACTORS PREDICTING ALLOGENEIC PERIPHERAL BLOOD STEM CELL (PBSC) MOBILIZATION AFTER G-CSF TREATMENT IN HEALTHY DONORS

Brissot, E.[1], Chevallier, P.[1], Guillaume, T.[1], Delaunay, J.[1], Ayari, S.[1], Saulquin, B.[1], Flandrois, G.[2], Devys, A.[2], Stocco, V.[2], Moreau, P.[1], Harousseau, J.L.[1], Mohty, M.[1] [1]CHU de Nantes, Nantes, France; [2]Etablissement Francais du Sang (EFS), Nantes, France

Some healthy donors may show poor mobilization response to G-CSF and poor CD34+ apheresis yields. Therefore, identifying donors at risk for poor mobilization could be of value in optimising transplantation approaches. This single centre report analyzed factors associated with PBSC mobilization and yield in a homogeneous caucasian population (n = 95; 53% males) of healthy adult donors. All donors received G-CSF dosed at 10 µg/kg/d for 5 days followed by large volume leukapheresis. 69 donors (73%) were healthy sibling donors, while 26 (27%) were healthy volunteer donors for HLA-identical unrelated transplants. All donors were undergoing their first PBSC mobilization. Donors' demographic characteristics were as follow (median; range): age, 47 (18–81) y., weight, 69 (43–106) kg, height, 170(150–187) cm, body-mass index (BMI), 23.9 (15.2–35.7) kg/m^2. As per institutional policy, the targeted total number of CD34+ stem cells was between 4 and 8 ×10e6/kg recipient body weight to be collected in a maximum of 3 apheresis sessions. Overall, the median number of collected CD34+ cells was 6.25 × 10e6/kg (range, 1.7–16.6), with 16 donors (17%) yielding less than 4 × 10e6/kg CD34+ cells. In univariate analysis, female gender, lower weight, height, pre-G-CSF and post G-CSF Hb levels, and low CD34+ cell counts prior to first apheresis, were associated with significantly lower total CD34+ stem cells yields (<6.25 × 10e6/kg). In multivariate analysis, male donor gender and higher post-G-CSF CD34+ cell counts prior to the first apheresis were most strongly associated with a higher total number of collected CD34+ stem cells (OR = 6.17, 95%CI (2.39–15.93), P = 0.0001; and OR = 3.95, 95%CI (1.53–10.19), P = 0.004 respectively). Also, when considering the group of 16 "poor" mobilising donors (CD34+ stem cells yield <4 × 10e6/kg), in multivariate analysis, we found that a higher post-G-CSF CD34+ cell count prior to the first apheresis was the strongest parameter significantly associated with a higher total number of collected CD34+ stem cells (OR = 6.36, 95%CI (1.68–24.15), P = 0.006). These results indicate that a quick assessment of risk for poor mobilization response in healthy donors can be achieved through simple demographic and routine parameters. Knowledge of predictive factors for mobilization to G-CSF may be of high interest, with the development of newer mobilizing agents like CXCR4 antagonists.

117

CORD BLOOD STORAGE FOLLOWING COLLECTION: IMPACT ON VOLUME REDUCTION AND CRYOPRESERVATION

Morin, H.[1], Wagner, E.[2], Champagne, M.A.[3] [1]CHU Sainte-Justine, Montreal, QC, Canada; [2]Centre Hospitalier Universitaire de Québec, Quebec, QC, Canada; [3]Hema-Quebec, St-Laurent, QC, Canada

Delays from cord blood (CB) collection to processing and cryopreservation vary among CB banks and optimal CB storage length and conditions are still poorly defined. Only about 25% of the collected CB units contain a total nucleated cell (TNC) content ≥1 × 10^9, providing enough cells to transplant a 40 kg recipient. Our preliminary results suggest that CB is best stored at 4°C for up to 3 days without significant losses in cell numbers and viability. We tested whether pre-cryopreservation CB storage conditions impact cell recovery and viability after processing and cryopreservation. CB units were collected and processed and cryopreserved either on the day of collection (day 0) or after a 3-day storage at 4°C or room temperature (RT). They were volume-reduced by buffy coat separation (Optipress II) or red cell sedimentation (Hespan). Processed CB units were cryopreserved and stored for 1 month before thawing and washing, and were assessed for TNC, mononucleated cell (MNC) and CD34+ cell recovery, and CD45+ and CD34+ cell viability. Storage at RT for 3 days induced a significant loss in TNC and MNC after processing whereas storage at 4°C did not induce a significant change compared to units processed on day 0. Both Optipress II- and Hespan-processed CB units showed low CD45+ cell viabilities when stored at RT (e.g. 64 ± 10% at RT and 82 ± 8% at 4°C *vs* 98 ± 2% on day 0, p<0.002 *vs* day 0 for the Optipress group). Interestingly, CB units stored at RT for 3 days showed reduced TNC, MNC and CD34+ cell recoveries and reduced CD34+ cell viability upon thawing and washing compared to units cryopreserved on day 0 or stored at 4°C for 3 days (e.g. CD34+ cell viability in the Optipress group: 70 ± 14% at RT, 87 ± 10% at 4°C *vs* 84 ± 5% on day 0, p<0,03 *vs* day 0). These results indicate that prolonged storage at RT prior to cryopreservation can negatively impact CB cell recovery and viability after processing and upon thawing and washing of cryopreserved units.

118

TREATMENT OF THREATENING REJECTION AFTER UMBILICAL CORD BLOOD TRANSPLANTATION WITH EX VIVO EXPANDED CB DERIVED T CELLS

Uhlin, M., Okas, M., Gertow, J., Ringden, O., Mattsson, J. Karolinska Institutet, Stockholm, Sweden

For patients lacking a human leukocyte antigen (HLA)-matched donor, umbilical cord blood (UCB) is a promising source of hematopoetic stem cells. Greater HLA disparity can be tolerated between the recipient and donor UCB compared with bone marrow or peripheral blood stem cells because of the naive and immature phenotype of UCB derived T cells. The risk of rejection is increased after UCB transplantation. After HLA-identical sibling or matched unrelated SCT the graft-versus-leukemia (GVL) effect may be increased by donor lymphocyte infusion (DLI) after SCT. However, after UCB transplantation DLI is not possible. This raised the question of whether ex vivo expanded CB lymphocytes (CBL) also can be used as a tool for adoptive immunotherapy after CB SCT. We have managed to establish a protocol for massive expansion of CB derived T cells making them usable in the clinic suitable for DLI after CB transplantation. We have further been able to show that the expansion protocol doesn't skew the T cell population regarding the phenotype, CD4:CD8 ratio as well as TCR usage profile measured by spectratyping. By activating the cells we have further investigated and confirmed the expanded T cells capacity to efficiently produce pro-inflammatory cytokines and respond in an allogeneic setting. We have now tried expanded CBLs in an adult patient with AML with threatening rejection after double UCB transplantation. The patient received Bu/Cy and ATG as conditioning therapy. He was transplanted with double UCB with a total nucleated cell dose of 5 × 107/kg. Both UCB units were 5/6 matched for HLA-A,-B and -DRB1 with low resolution HLA-typing. Chimerism analysis one month after transplant showed mixed chimerism in T-, B- and myeloid cells of both donor units and recipient. At four months after transplant the patient had 85% recipient cells in both T cells and myeloid cells indicating a threatening rejection. However, in bone marrow CD34+ cells were 90% of donor origin. Due to anticipated rejection the patient was treated with expanded CBLs, 5 × 103/kg at 4 months, 1 × 104/kg at 5 months and 1 × 105/kg at six months. Immunusuppression was tapered at six months. The immunemodulatory treatment was well tolerated with no development of GVHD. So far no change in chimeric pattern has been shown. The immunemodulatory treatment will be continued with increasing doses of CBLs.

119

STANDARDIZATION OF T CELL (CD3+) DOSE FOR ALLOGENEIC PERIPHERAL BLOOD STEM CELL GRAFTS

Innis-Shelton, R., Tilden, A., Ashraf, K., Vaughan, W.P., Lamb, L.S. University of Alabama at Birmingham, Birmingham, AL

The number of CD3+ cells collected from donors varies widely and no significant attempts have been made to standardize the graft T cell content within the different categories of stem cell sources. Preliminary data from our center have shown that that administration of dexamethasone during the last three days of G-CSF mobilization reduces the donor T cell content of the graft by approximately 0.5 log, also resulting in a significant decrease in the incidence and severity of acute GvHD. Based on these findings, we have developed a procedure to standardize the graft to deliver a PBSC product with a T cell dose of 3.0 ± 0.5 × 10^7 CD3+ cells/kg recipient body